Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsarkitektur, trädgårds- och växtproduktionsvetenskap

Transgenerationell fenotypisk plasticitet: kan föräldrars erfarenhet påverka avkommans fenotyp?

– En studie i beteende och fysiologi hos *Spodoptera littoralis*

Transgenerational phenotypic plasticity: can parental host plant experience affect the phenotype of the offspring?
– A study in behavior and physiology in *Spodoptera littoralis*.

Axel Rösvik

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Abstract

The Darwinian theory of natural selection first popularized by Charles Darwin in the mid-19th century has been widely accepted as one of the key mechanisms of evolution. However, before Darwin other scientists such as Jean-Baptiste de Lamarck had their theories about evolution. Lamarck's theory of inheritance of acquired characteristics was one of these theories and it has today been reexamined as transgenerational epigenetics. In this study we will investigate in the polyphagous moth *Spodoptera littoralis* (Lepidoptera: Noctuidae) the potential for transgenerational epigenetic inheritance, or transgenerational phenotypic plasticity (TPP), as it will be referred to in this thesis. It is known that parental experience can alter the phenotype of the offspring and therefore increase its fitness in the environment experienced by the parent. In this study, we will test if parental plant experience can affect the behaviour and physiology of second-generation individuals of *S. littoralis*, in preference, migration and performance bioassays. We found evidence of parental effect in the performance of offspring reared on the same diet as the parent. Interesting trends in the behaviour were also found but no significant difference showing transgenerational modulations of offspring behaviour were shown. Further studies are needed to understand the mechanisms underlying TPP in *S. littoralis*.

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Introduction

Transgenerational phenotypic plasticity

Evolutionary theories such as the theory of natural selection that was first popularized by Charles Darwin in the mid-19th century have until today been widely accepted as one of the key mechanism of evolution (Çabej 2012). However, Darwin himself wrote in the introduction of his first work On the Origin of the Species: ”I am convinced that Natural Selection has been the main but not exclusive means of modification.” (Darwin 1859, p 6).

Darwin was referring to the theory of “inheritance of acquired characteristics” of Jean-Baptiste de Lamarck (1809). Today, approximately 156 years after Darwin’s first publication, the phenomenon of transgenerational epigenetic inheritance is considered to be an important mean of inherited modifications (Çabej 2012). In this study we will refer to these inheritable traits as transgenerational phenotypic plasticity (TPP) since we are not investigating the underlying mechanisms. In other words, we don’t investigate whether these mechanisms are epigenetic or work through other transgenerational means such as chemical imprinting as described in Quesada & Schausberger (2012).

TPP is the ability of a parent to change its offspring’s phenotype transgenerationally and therefore increase its fitness in the experienced environment of the parent (Cahenzli & Erhardt 2013). This sudden change in offspring phenotype involves no genetic change in the organism and can therefore allow a rapid response to environmental change (Çabej 2012; Richter-Boix *et al*. 2014). Recent studies are indicating that TPP is working in a number of different ways through quantity and composition of hormones, enzymes, proteins, mRNA and environmental contaminants (Mousseau & Fox 1998; Faulk & Dolinoy 2011; Meylan 2012; Sheriff & Love 2013).

TPP is also believed to be one of the underlying mechanisms of the rapid speciation that is occurring in nature. An example from the insect world is the ongoing speciation of the *Drosophila* genus that is unexplainable through the neo-Darwinian theories (Çabej 2012). Unfortunately most of the TPP studies have been done on vertebrates and it is therefore poorly documented in insects (Quesada & Schausberger 2012; Cahenzli & Erhardt 2013).

Model organism

The organism used for this study is the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). *S. littoralis* is a highly polyphagous agricultural pest that is widespread in Africa, Middle East and locally established in the southern parts of Europe (Brown & Dewhurst 1975; Holloway 1989; CABI & EPPO 2011). Its host range spans 87 species of economic importance in the regions where it is present (Salama *et al*. 1970). Crops such as cotton (*Gossypium sp*.), maize (*Zea mayz*), cowpea (*Vigna unguiculata*) and potato (*Solanum tuberosum*) can be completely stripped by the phytophagous larvae (CABI & EPPO 2011).

Host plant preference, larval migration and performance of *S. littoralis*

When the adult female of *S. littoralis* is choosing a host plant for oviposition she is doing a very important choice for the upcoming generation. The oviposition choice of the mother, in the case of a polyphagous species such as *S. littoralis*, literally determines the fitness and survival of the progeny and therefore it is important that the mother chooses a suitable host-plant for her offspring (Anderson & Anton 2014; Schoonhoven *et al.* 2005). The adult female is able to discriminate between different host plants and according to previous studies *S. littoralis* has an innate preference hierarchy when it comes to host plant choice (Anderson & Anton 2014). This preference hierarchy can be influenced by earlier experiences in the larval stage of the insect and then passed through metamorphosis and affect the oviposition behavior of the adult female (Anderson *et al.* 2013; Thöming *et al*. 2013; Çabej 2012). According to Valladares & Lawton (1991) this so called "mothers knows best" theory makes the adult female able to make a choice relying on her innate preferences and therefore choosing a suitable host plant were the offspring will have a high fitness.

The offspring larvae, like their mother, also seem to have an innate preference hierarchy (Khallaf 2015, unpublished data). However, the host plant the larva is emerged on directly induces a preference in the naïve larvae since the larvae tend to feed on the young and nutritious leaves on the plant on which it hatched before migration occurs (Sadek 2011). If the mother chooses an unsuitable host plant the neonate larvae are capable of leaving the plant in search for a more suitable food source despite their limited energy reserves (Schoonhoven *et al.* 2005). This migration behavior of the larvae is usually occurring naturally after a few days. The larvae then tend to disperse and migrate within and beyond the plant to reduce

intraspecific competition and maximize their performance (Sadek 2011). Therefore it is also important for the neonate larvae to be able to assess plant stimuli and discriminate between plants to be able to find a suitable host plant when they are ready to migrate (Schoonhoven *et al.* 2005).

Previous studies on the predatory mite, *Neoseiulus californicus*, by Quesada & Schausberger (2012) have shown that parental diet can affect feeding preferences of the offspring. In this study, the predatory mites were introduced to prey fed on different diets and the subsequent offspring tended to choose the prey fed on the same diet as the mother had been fed with. It is believed that the chemical remains of the gravid females diet is incorporated into the egg and therefore affecting the offspring's preference. This prenatal chemosensory learning has been previously documented in mammals, birds, amphibians, molluscs and fish but not in arthropods (Quesdada & Schausberger 2012). If this would be the case in *S. littoralis* there might be a chance that this would be visible in preference and migration bioassays.

Another study on TPP in arthropods by Cahenzli & Erhardt (2013) shows that the performance of the butterfly, *Coenonympha pamphilus* (Lepidoptera: Nymphalidae), is increased in terms of pupal weight depending on parental diet. The offspring of parents reared on either high- or low nitrogen containing food gets physiologically acclimatized to the diet of the same nitrogen content as the parent and therefore performs better on this diet. The weight of the pupae can be directly translated to the size of the adult that then can be directly correlated with spermatophore size of the male and potential fecundity of the female (Thornhill 1976; Honěk 1993). Other means of performance is of course survival and time from larval emergence until pupation. Survival and developmental time from emergence until pupation is a direct effect from the ability to utilize a food source in holometabolous species (Allegret 1964; Hagstrum & Milliken 1988). Previous studies have shown that symbiotic microorganisms can be transferred from parent to offspring. These microorganisms could then prepare the progeny for a certain diet by providing essential nutrients and detoxifying secondary metabolites and therefore increase its development on the host (Brooks 1963 see Mousseau & Fox 1998; Spitzer 2004).

Objective

The aim of this study is to investigate if parental host plant experience can alter the phenotype of the offspring in the polyphagous moth *S. littoralis*. The alterations we are looking for are difference in plant preference, migration tendency and performance in the offspring of parents reared on either maize or cotton. Indications of TPP occurring in this species should be visible using a cross-fostering design in which larvae will be reared in either their own or unrelated parental environment.

Materials and Methods

Plants

Maize plants (*Zea mays* variety Sweet Nugget), and cotton plants (*Gossypium hirsutum* variety Delta Pineland 90), were cultivated from seed at SLU Alnarp.

Plants used for the bioassays were cultivated in 1.5L round plastic pots (Ø =15cm) in commercial substrate Röd Kronmull (Weibull trädgård AB, Hammerhög, Sweden) for four to six weeks at 25°C ± 2°C, 75 % ± 2 % RH with artificial light (Osram Powerstar HQI-T, 400W/D, 16:8 h LD cycle).

Insects

The insects were reared at SLU Alnarp in a controlled climate chamber at 25°C ± 2°C 65 % ± 2 % RH with a 17:7 LD cycle.

Parents of larvae used for the bioassays were reared on either of two different plants, maize or cotton, from first instar until pupation. This generation is referred to as the parental generation (F0). The adults of the parental generation (F0) were then put into cages containing the same plant as the individuals had experienced in the larval stage. Both males and females were put in the cage for mating to occur. The eggs (F1) collected from both plants and cage walls were then carefully scraped of the surface on which they were oviposited and placed in petri dishes for hatching. Larvae used for the preference bioassay were reared on artificial diet as described in Hinks & Byers (1976). All larval experiments were carried out in a climate chamber at 25°C, RH 50%, 12:12 LD cycle.

Performance bioassay

Larvae from the second generation were put into separate plastic cups (30ml) containing plaster to keep humidity. The larvae were then fed every other day from first to third instar and then daily from third instar until pupation. Food was always present so that there would be no biases due to larvae not being able to feed. Offspring from parents reared on cotton and maize were either fed on same diet as the parent or switched to the plant not experienced by the parent (Fig. 1). This was done to be able to do a cross assessment on their performance between the different diets depending on the diet of the parent. The following traits were monitored:

1. Mortality
2. Pupal mass
3. Larval development time
4. Growth rate

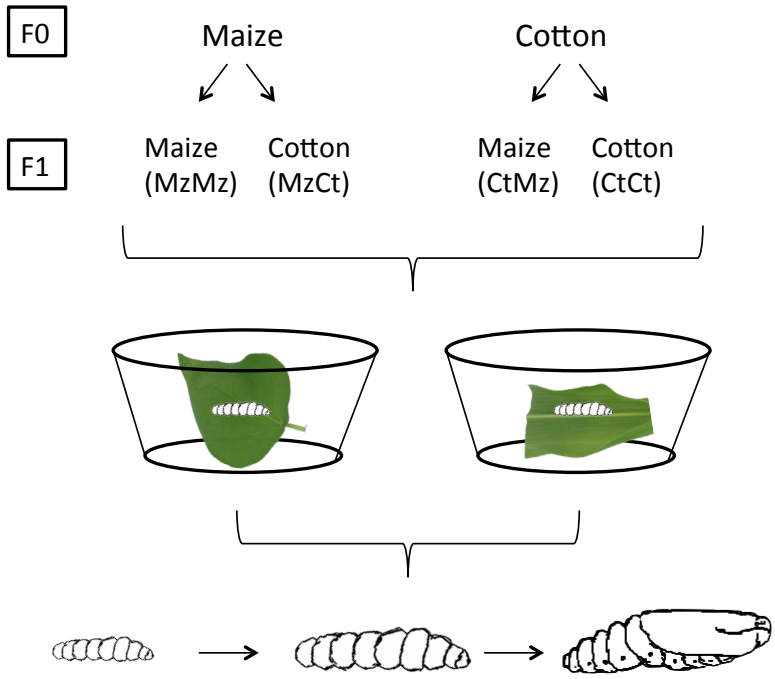

Figure 1. Schematic illustration of performance bioassay (first two letters in parenthesis indicates the diet of the parent and last two letters indicates the diet of the offspring. Ct=Cotton, Mz=Maize; CtCt n=37, CtMz n=39, MzCt n=39, MzMz n=39).

Due to an aphid invasion in the greenhouse, which led to a food shortage, the replicates had to be cut from 70 to 40. This was done during the 4th instar. The replicates removed were randomly chosen with a Microsoft Excel based number generator.

The larvae were checked every morning to see if pupation had occurred. The pupae were transferred to a plastic cup (30ml) without plaster and put in an incubator at 17°C to delay the emergence of the adult. The pupae were kept in the incubator for two weeks. This was done because of the shortage of plant material for the continuing migration bioassay. The pupae were weighed 24h after pupation had occurred using an A&D HR-200 laboratory scale.

Preference bioassay

1st instar larvae from parents reared on cotton or artificial diet (control) were put on a petri dish with two leaf discs (Ø=0.5cm) from maize and cotton with a 5cm interspace (Fig. 2). The larvae then had four hours to make a choice between either the maize- or cotton disc. The petri dishes were observed during these four hours to make sure that the first choice of the larva was noted.

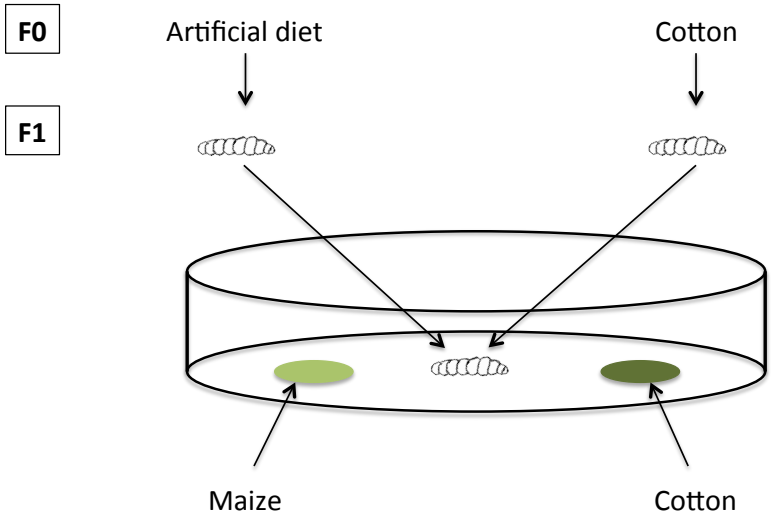

Figure 2. Schematic illustration of the preference bioassay (Control n=110, Cotton n=116).

Migration bioassay

20 newly hatched 1st instar larvae (F1) of parents reared on cotton or maize were put in plastic boxes containing water filled vials with cotton- and maize leafs. The larvae of parents reared

on cotton and maize were either put on the same plant experienced by the parent or switched to the plant not experienced by the parent to be able to do a cross assessment between the treatments (Fig. 3). Larvae were then left for three days and on the third day both leaves were removed from the box and the numbers of migrated larvae were counted.

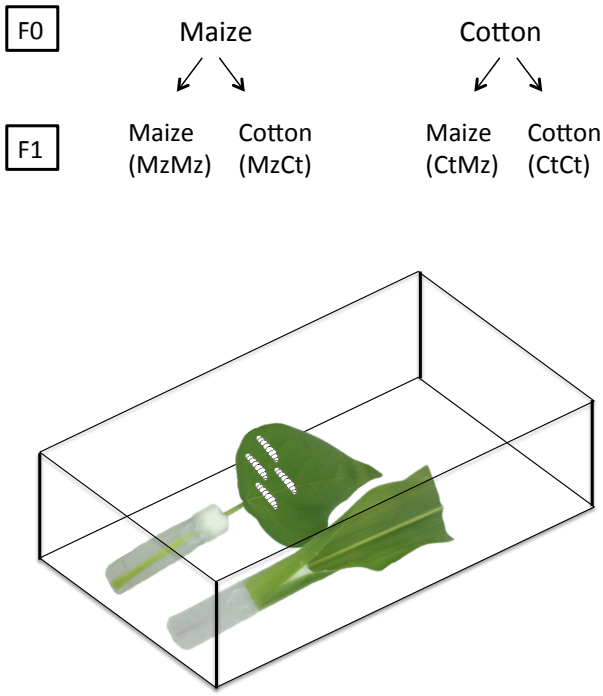

Figure 3. Schematic illustration of migration bioassay (first two letters in parenthesis tells the plant which the parent have been exposed to and last two letters tells the leaf the offspring was put on. Ct=cotton, Mz=Maize; CtCt n =10, CtMz n=9, MzCt n=10, MzMz n=10).

Statistical analysis

The statistical analyses were performed using R statistical software (R Core Team 2014) and Minitab 16.2.4 statistical software.

Performance bioassay

Following normality- and analysis of variances tests were carried out: Gaussian function, Poisson distribution and Shapiro-Wilk test.

Tukey's range tests were performed to compare the observed traits between the treatments. The pupal mass, larval developmental time and growth rate data were analysed

using R statistical software. To calculate the rate at which the larvae grew on a daily basis the pupal mass were divided by the days it took for the larvae to pupate according to the following formula:

$$Development\ rate\ (\frac{mg}{d}) = \frac{Pupal\ mass\ (mg)}{Days\ to\ pupation\ (d)}$$

Mortality of the larvae was calculated in two steps. First step was from 1st instar to 4th instar and the second step was from 4th instar until pupation. This was done because there was a cut in replicates approximately two weeks after the experiment started due to lack of plant material for feeding. Two sample t-tests were performed to compare the mortality between the treatments.

Preference bioassay

A preference index was calculated from the preference data collected. This preference index was calculated with the following formula:

$$Preference\ index = \frac{(Cotton - Maize)}{(Cotton + Maize)}$$

Wilcoxon signed rank tests were then performed against the zero value to see if there was any preference to either cotton or maize within the treatments.

Wilcoxon signed rank tests were also used to compare the treatments with each other to see if there was any difference between the treatments.

Migration bioassay

A migration index was calculated from the migration data collected. This migration index was calculated with the following formula:

$$Migration\ index = \frac{Migrated\ larvae}{Total\ number\ of\ larvae}$$

Data were compared by performing a generalized linear model (binomial error, log link function). We tested for plant effect, parental effect and the interaction between these two

variables. The batch effect was also tested and treated as random variables. GLM was performed using an ANOVA and chi-square test as test criterion. Post hoc pair-wise comparisons were made using Tukey's contrasts (multicomp package in R).

Results

Parental effect on larval performance

Mortality

Mortality between the treatments from 1st to 4th instar (Fig. 4) were: MzMz 10 %, CtMz 11.4 %, CtCt 5.7 % and MzCt 1.4 %. The two sample t-tests carried out between the treatments showed significant difference between treatments MzCt x CtMz (P=0.01) and MzMz x MzCt (P=0.03). The number of replicates within every treatment from 1st to 4th instar is 70. Mortality between treatments from 4th instar until pupation (Fig. 5) were: MzMz 2.5 %, CtMz 2.5 %, CtCt 7.5 % and MzCt 2.5 %. No significant differences were found between any of the treatments: MzCt x CtMz (P=1.0), MzMz x MzCt (P=1.0), MzMz x CtCt (P=0.31), MzCt x CtCt (P=0.3), CtMz x CtCt (P=0.31) and MzMz x CtMz (P=1). The number of replicates within every treatment from 4th instar until pupation is 40.

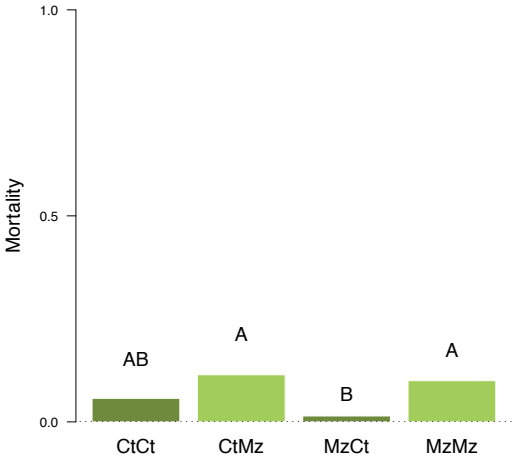

Figure 4. Mortality of larvae from 1st to 4th instar (1=100% mortality and 0=0% mortality). Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Different letters indicate significant difference (t-test, P<0.05).

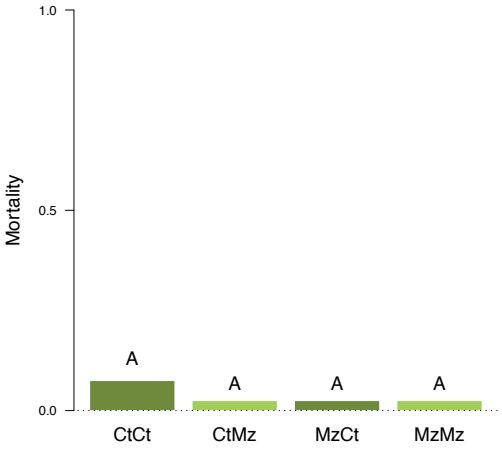

Figure 5. Mortality of larvae from 4th instar to pupation (1=100% mortality and 0=0% mortality). Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Different letters indicate significant difference (t-test, $P<0.05$).

Pupal weight

Significant difference in pupal weight was found between the larvae reared on different plant material in the offspring generation (F1) irrespective of parental diet (F0) (Fig. 6. $P < 0.001$) in comparisons CtMz x CtCt, MzMz x CtCt, MzCt x CtMz, MzMz x MzCt. In the case of the offspring reared on same diet there was no significant difference between the treatments on offspring (F1) reared on maize MzMz x CtMz (Fig. 6. $P = 0.97$). In offspring reared on cotton there was a difference between the treatments MzCt x CtCt (Fig. 6. $P=0.016$)

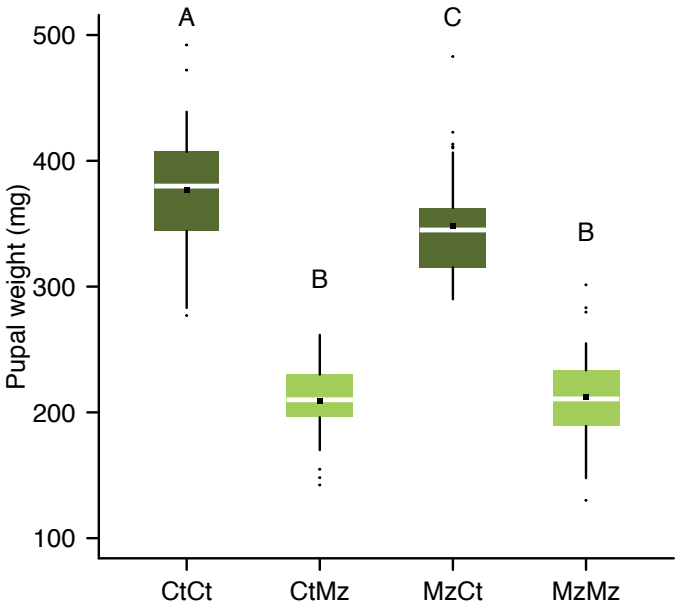

Figure 6. Pupal weight of larvae of *S. littoralis*. Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Box plots show the mean (black square), median (white line) and 25–75% percentiles. Whiskers show all data excluding outliers. Outliers (dots) are values being more than 1.5 times box length from upper and lower edge of respective box. Letters indicate significant differences between treatments (GLM, Tukey's test, $P<0.05$).

Time of pupation

Significant difference in development time was found between larvae reared on different plant material in three cases CtMz x CtCt (Fig. 7. $P=0.03$), MzMz x CtCt (Fig. 7. $P<0.001$) and MzMz x MzCt (Fig. 7. $P<0.01$). No significant difference between the treatments was found between the larvae reared on the same plant material MzCt x CtCt (Fig. 7. $P=0.99$), MzMz x CtMz (Fig. 7. $P=0.71$). In one case there was also no significant difference in development time between larvae reared on different plant materials MzCt x CtMz (Fig. 7. $P=0.05$).

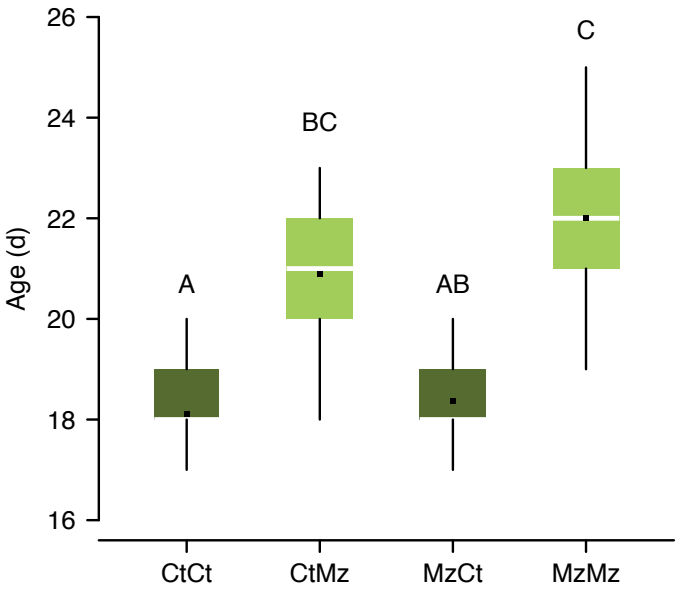

Figure 7. Developmental time to pupation (d) of larvae of *S. littoralis*. Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Box plots show the mean (black square), median (white line) and 25–75% percentiles. Whiskers show all data excluding outliers. Outliers (dots) are values being more than 1.5 times box length from upper and lower edge of respective box. Letters indicate significant differences between treatments (GLM, Tukey's test, $P<0.05$).

Growth rate

Significant difference in growth rate was found between the larvae reared on different plant material in the offspring generation (F1) irrespective of parental diet (F0) (Fig. 8. $P< 0.001$) in comparisons CtMz x CtCt, MzMz x CtCt, MzCt x CtMz and MzMz x MzCt. No significant difference was found in offspring (F1) reared on maize MzMz x CtMz (Fig. 8. $P=0.09$). In offspring reared on cotton there was a difference between the treatments MzCt x CtCt (Fig. 8. $P<0.01$).

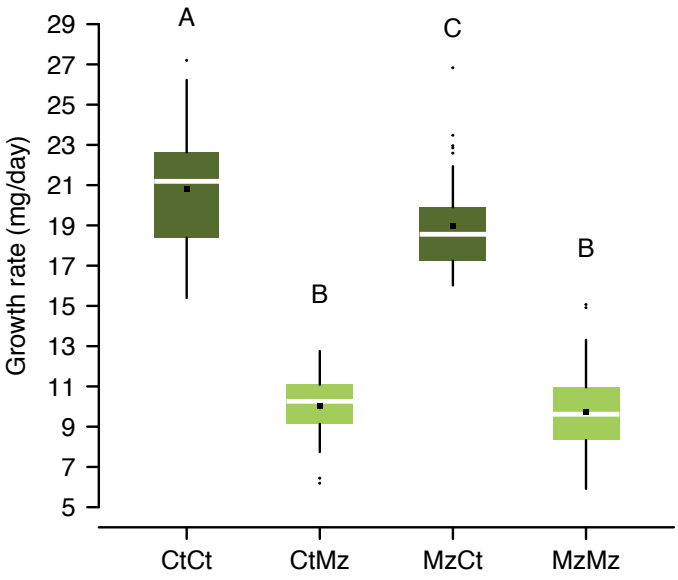

Figure 8. Growth rate of larvae of *S. littoralis* from 1st instar to pupation. Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Box plots show the mean (black square), median (white line) and 25–75% percentiles. Whiskers show all data excluding outliers. Outliers (dots) are values being more than 1.5 times box length from upper and lower edge of respective box. Letters indicate significant differences between treatments (GLM, Tukey's test, $P<0.05$).

Preference bioassay

No significant preference for either maize or cotton within treatments for either offspring from parent fed on artificial diet (Fig. 9. P=0.25) or offspring from parent fed on cotton (Fig. 9. P=0.25). No significant difference between the two treatments (Fig. 9. P=0.057).

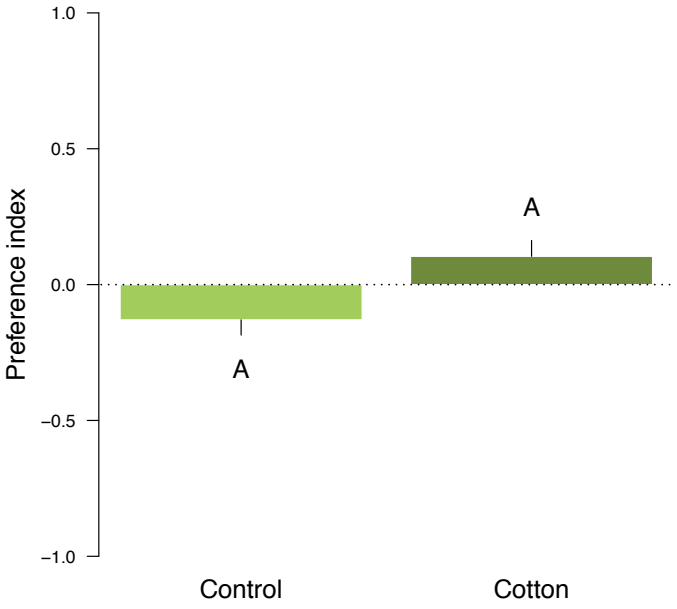

Figure 9. First instar larvae preference between cotton (>0) and maize (<0). Control larvae come from parents reared on neutral media and not exposed to plant odour (light green). Treated larvae come from parents reared on cotton and exposed to cotton odour as adults (dark green) (GLM, Tukey's test, P<0.05).

Migration bioassay

The statistical tests showed no parental effect on migration (Fig. 10. P=0.065) and no significant difference between the interaction of parental- and plant effects (Fig. 10. P=0.75). No batch effect between the egg batches collected could be found (Fig.10. P=0.083). However, a clear plant effect could be seen (Fig.10. P<0.001).

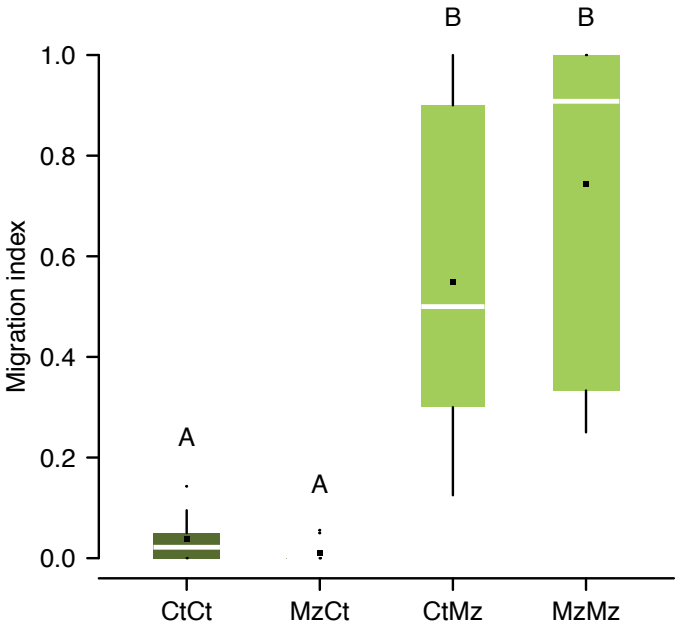

Figure 10. 1st instar larvae migration tendencies (1 is full migration and 0 is no migration from plant). Larvae come from parents reared on cotton and kept on the same diet (CtCt) or switched to maize (CtMz), or from parents reared on maize and kept on the same diet (MzMz) or switched to cotton (MzCt). Box plots show the mean (black square), median (white line) and 25–75% percentiles. Whiskers show all data excluding outliers. Outliers (dots) are values being more than 1.5 times box length from upper and lower edge of respective box. Letters indicate significant differences between treatments (GLM, Chi-squared test, $P<0.05$).

Discussion

Preference

In the results of the two-choice bioassay the larvae from the control and cotton treatments showed no significant preference for either cotton or maize. There's no significant difference between the two treatments either. This indicates that there's no parental effect on the offspring preference. However there seems to be a trend towards a preference for cotton for the offspring from cotton-exposed parents and a trend towards maize from the offspring from artificial diet reared parents with no previous exposure to cotton.

In its natural habitat the females of *S. littoralis* have a wide host range to choose from for oviposition. In this species, host plant choice is guided by an innate host-plant preference that can be modulated by early larval or adult experience (Thöming *et al.* 2013; Proffit *et al.* 2015). The larvae of *S. littoralis* also seem to exhibit an innate host plant preference (Khallaf 2015, unpublished data). However, larvae usually exhibit an induced preference for the plant

that they emerged on (Sadek 2011). In this bioassay the eggs of the parent exposed or non-exposed to cotton were scraped off their oviposited surface and put on a petri dish for hatching. Therefore when the larvae hatched they were not exposed to any host plant and would therefore solely rely on innate preference or preferences induced by TPP.

In the study of Quesada & Schausberger (2012) on *Neoseiulus californicus* (Mesostigmata: Phytoseiidae), the offspring's feeding preferences are directly affected by the parental diet. If this would be the case of *S. littoralis* there would have been indications on this in the results. However, in this bioassay, the first instar larvae are not even able to show any innate preferences since they don't seem to prefer one host plant to the other.

Another effect that could possibly affect the preference of the larvae is the "egg imprinting" effect as described by Karpinski *et al.* (2013). In this study it was shown that adults of *Heliothis virescens* (Lepidoptera: Noctuidae) tend to oviposit on the plant that they were themselves oviposited on instead of the plant on which they were fed. This indicates that the preference in this case is induced in the egg stage and not the larval stage. However, it is unknown if this chemical imprinting effect of the egg could induce a preference in the larvae. Although, as mentioned earlier in the study by Quesada & Schausberger (2012), prenatal chemosensory learning is in this case altering the preference of the offspring in *N. californucus* and might work similar to the egg imprinting effect as described by Karpinski *et al.* (2013).

The offspring might also only rely on their mother when it comes to her choosiness in host plant choice for oviposition. The preference-performance hypothesis (Jaenike 1978), or 'mother's know best' (Valladares & Lawton 1991) as it is also called, states that the female will oviposit on a suitable host that minimizes mortality and maximizes performance of the offspring (Clark *et al*. 2011). If this is the case the 1st instar larvae of *S. littoralis* might not have any strong innate preference and the latter preference might therefore be postnatally induced when exposed to odour cues from host plants (Carlsson *et al*. 1999).

Migration

In the migration bioassay there was no batch, parental or parental x plant interactive effect between the treatments. However, there was a plant effect between the treatments. The plant preferred by the first instar larvae seemed to be cotton independent of parental diet. Larvae put on cotton tended to stay on the leaf and larvae put on maize tended to migrate from the maize leaf to the cotton leaf.

For the first instar larvae it is crucial to find a suitable food source soon after emergence to be able to survive. The larvae therefore have to regard the food sources available and negotiate which is the most suitable host (Zalucki *et al*. 2002; Schoonhoven *et al*. 2005). Plants differ in both mechanical and chemical structure, which can have a negative effect on the first instar larvae (Chapman & Bernays 1989; Lucas *et al*. 2000). Mechanical structures in plants such as amorphous silica can tear on the mandibles of the neonate larvae and might therefore affect feeding (Lucas *et al*. 2000). In the case of maize and cotton there might be chemical or mechanical structures that make the neonates discriminate against maize in the selection process and therefore consider cotton a more suitable host and therefore lead to a migration to the cotton leaf (Schoonhoven *et al.* 2005).

This migration bioassay is similar to the first preference bioassay that was done. However in the preference bioassay the larvae seemed unable to make a choice between cotton and maize leaf disc. In the migration bioassay the larvae didn't have any problem to discriminate between the plants even though the larvae were put directly on the leaf. The fact that bigger and more intact leaves were used instead of leaf discs and the larvae were left for three days could affect the outcome of the bioassay. Also the fact that 20 larvae per bioassay were used could have affected the results.

In its natural environment the female of *S. littoralis* lays egg batches of 100-300 eggs (Miyahara *et al*. 1971). This results in hundreds of larvae aggregating at hatching. The neonate larvae tend to feed together for the first instars before aggregation breaks up (Common 1990). This might therefore lead to larvae following each other and making a "group" decision in the migration bioassay. This is not the case in the preference bioassay since the larva is alone on the petri dish. However, the actual mechanisms used by the neonate larvae to find a suitable host are not well studied and researchers seem to avoid working with first instar larvae since they are inconvenient to handle (Zalucki *et al*. 2002).

Performance

In the performance bioassay we showed a clear plant effect when it comes to mortality and developmental time from 1st instar to pupation. No parental effects were found for these traits. We also found a plant effect and parental effect on the pupal weight. The offspring reared on cotton with parents reared on cotton had a significant increase in pupal weight compared to offspring from parents reared on maize. This was not the case in the offspring reared on maize were the pupal weight between offspring from parents either on maize or cotton didn't differ

significantly. Since there wasn't any significant difference in the developmental time from 1st instar to pupation depending on parental diet the factor differentiating the growth rate is solely the pupal weight in this case.

The reason there's a difference in mortality between offspring reared on cotton and maize can be explained by the mechanical and chemical structure of the plant and therefore its suitability as a host plant (Chapman & Bernays 1989; Lucas *et al*. 2000). The significant difference in mortality was only visible in the first larval instars and could presumably be explained by plant defensive obstacles such as amorphous silica explained by Lucas *et al.* (2000). In the later instars the mortality decreases except for treatment CtCt. Some of the larvae in this treatment seem to have been infected with a virus, supposedly Baculovirus, and thus leading to an increased mortality even in the later instars (Funk 1997; El-Sheikh & Ashour 2011)

The offspring reared on cotton seems to have been able to develop and pupate faster than the offspring reared on maize independent on the parental diet. This could again be explained by the suitability of the host plant as a food source for the larvae. The higher pupal weight of the individuals fed on cotton also tells us that the larvae can utilize this food source easier than maize and therefore gain a higher pupal mass which then can be directly correlated with adult fitness of the individual (Hagstrum & Milliken 1988; Honěk 1993). However, there was a significant difference in pupal weight between the larvae reared on cotton with parents on different diets. This means that in some way the offspring from parents reared on cotton, which had a higher pupal mass, were transgenerationally affected by the parental diet as in the case of *C. pamphilus* in the study by Cahenzli & Erhardt (2013) were offspring from parent reared on food sources containing either low or high nitrogen content seemed to perform better on the food with the same nitrogen content as the parent. One explanation to the difference in pupal weight could be that there's a microbial flora that can provide essential nutrients or detoxify plant defensive compounds that is transferred from parent to offspring and therefore prepare the offspring for the experienced environment of the parent (Brooks 1963 see Mousseau & Fox 1998; Spitzer 2004). The idea of offspring performing better on the experienced plant of the mother could lead to a higher fitness for this plant over generations and lead to the start of a host-race formation, which in the long run is believed to lead to sympatric speciation (Mousseau & Fox 1998; Spitzer 2004; Berlocher & Feder 2002; Cahenzli & Erhardt 2013). One must also consider that a natural selection process occurring in the parental generation could explain the difference in pupal weight between the offspring on cotton. Since first generation was reared together in a plastic container the mortality could

not be monitored and therefore we can't disregard a selection process occurring that could influence the outcome of the result. However, considering the very low mortality rate we observed in our experiments (Fig. 4 and 5), it is unlikely that selection played a role in shaping the physiology or behavior of the larvae.

Conclusion

In the case of *S. littoralis* there seems to be TPP influencing the physiology of the organism and in this way giving the offspring an increased fitness in the experienced environment of the parent. However, the behavior of *S. littoralis* doesn't seem to be transgenerationally influenced in offspring from parents reared on a specific diet. According to the mother's know best theory the mother oviposits on the most suitable host to reduce mortality of the offspring therefore introducing a host plant for the offspring. The offspring in this case would therefore not be in need of any change in preference and solely be relying on the mother. However, more studies on TPP and general knowledge about the mechanisms behind TPP are needed.

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